U. S. FOREST SERVICE RESEARCH NOTE NE-39

1965

Northeastern Forest

FOREST SERVICE, U.S. DEPT. OF AGRICULTURE, 102 MOTORS AVENUE, UPPER DARBY, PA.

Experiment Station

INSECT-INDUCED CRYSTALLIZATION OF WHITE PINE RESINS. II. WHITE-PINE CONE BEETLE

The white-pine cone beetle (*Conophthorus coniperda* (Schwarz)) can cause extensive damage to cones of eastern white pine (*Pinus strobus* L.) and can severely hamper natural reproduction of this species (Graber 1964). This insect also will be a potential pest of seed orchards for the production of genetically superior seed if and when such orchards are established. It may be assumed that in certain areas, and for certain purposes, these seed orchards will contain white pine species that are exotic to the Northeast, or are hybrids of *P. strobus* and exotic species. Knowledge about the relative vulnerability of non-native species to this insect is extremely limited.

It was noted, when dissecting beetle-infested conelets, that the resin appeared to be crystallized into small globules throughout the conelet. Several recent studies (Harris 1960; Yates 1962; Santamour 1965) have indicated that attacking insects may induce resin crystallization. Because of the possibility that the observed crystallization was caused by the insect and that differences in resin crystallization may be indicative of relative resistance to insect attack, a study was undertaken to determine the effect of the cone beetle on crystallization of the resins of a number of white pine species and hybrids.

Oleoresin from cortical canals of young shoots appeared to be similar to that found in the cortex of conelet peduncles, and therefore shoot oleoresin was used in this study. Both cortical resins were incompletely soluble (white flocculent material settled out) in methanol, ethanol, and acetone; whereas wood (xylem) resin was completely soluble (Santamour 1965).

Materials and Methods

Oleoresin was collected in April 1964 as it exuded from cortical resin ducts of freshly-cut 1-year-old branches. Generally this collection was made with an attenuated eye-dropper, but in certain free-flowing species the resin was simply allowed to drip into the collecting vial. Resin was stored in screw-capped glass vials at room temperature. One vial of each resin sample was left undisturbed for study of natural crystallization; resin from the other vial was used in the tests described below within 3 days after collection.

The trees from which oleoresin was collected included native eastern white pine from New Hampshire and a number of exotics and hybrids growing in plantations of the Northeastern Forest Experiment Station and the Cabot Foundation of Harvard University. The kind permission of the Cabot Foundation to use its exceedingly valuable material is gratefully acknowledged.

Conelets of native white pine that were infested with the cone beetle were collected in November 1963 from the ground beneath an open stand of this species in New Salem, Massachusetts. The conelets were stored in a paper bag in a refrigerator. Conelets were broken apart manually to collect the beetles, and only those beetles that were alive and uninjured were used in the experiments.

Tests of insect-induced crystallization were made by macerating the severed head and thorax of the adult beetle in a drop of resin on a clean glass slide. The slide was then incubated at 100° F. and test reactions were determined after 4 hours. Slides bearing a drop of untreated resin were included in each test as controls. In addition, the whole live beetle, macerated abdomen, and frass from fresh attacks were used in resin of *P. strobus* and *P. griffithii* McClel. (Himalayan white pine). A positive test was one in which the resin crystallized into a hard, white, dry mass within 4 hours.

Several tests of beetle attack were also made in the laboratory. For these tests, freshly-cut pine branches bearing 1-year-old conelets were placed with their bases in water, and healthy adult beetles were liberated in the area around the conelet.

The beetles were not separated by sex for either the crystallization tests or the attack studies. Godwin and ODell (1965) found that although males did not initiate attacks in the spring, young males that had not overwintered were as likely to initiate attacks in the summer as were young females. The male-female sex ratio (1:2) found by Godwin

and ODell in overwintered conelets would suggest that more females than males were used in the present tests.

Results and Discussion

The results of crystallization tests of macerated beetle heads (and thoraxes) on shoot resins of various white pine species and hybrids are given in table 1. Generally, only one tree of a given species or hybrid was used in the tests. Exceptions were *P. strobus* (6 trees); *P. peuce* Griseb. (Macedonian white pine) (2 trees); and *P. peuce* X *strobus* (5 trees).

Macerated head and thorax, macerated abdomen, whole live beetle, and fresh frass were equally effective in inducing crystallization of *P. strobus and P. griffithii* resin. However, crystallization induced by whole beetles was limited to the area around the beetle.

All the resins that crystallized in the presence of cone beetles—with one exception—also crystallized during undisturbed storage. Yates

Table 1.—*Occurrence of natural and insect-induced crystallization of white pine shoot resins*

Species or hybrid, *Pinus*—	Natural crystallization	Test crystallization
Series Cembrae:		
koraiensis	0	0
Series Flexiles:		
flexilis	0	0
reflexa	0	0
armandi	0	0
Series Strobi:		
ayacahuite	+	+
griffithii[1]	+	+
lambertiana[1]	0	0
monticola	0	0
parviflora	+	+
peuce[1] (2 trees)	0,0	0,0
strobus (6 trees)	+,+,+,+,+,+	+,+,+,+,+,+
ayacahuite X O.P. (probably *monticola*)	0	+
ayacahuite X *strobus*	+	+
peuce X *griffithii*	0	0
peuce X *strobus* (5 trees)	0,0,+,+,+	0,0,+,+,+
monticola X *parviflora*	+	+
monticola X *strobus*	+	+
strobus X *griffithii*	+	+

[1]Grafted on *P. strobus*.

[2]Female parent is listed first in hybrid combinations.

(1962) and Santamour (1965) have previously pointed out that resins that have a tendency toward natural crystallization also crystallize more readily in the presence of insects. The one exception to this generalization was a single individual of an open-pollinated progeny of *P. ayacahuite* Ehrenb. (Mexican white pine) that, upon morphological examination, was determined to be a hybrid with *P. monticola* Dougl. (western white pine). The relationship between natural and induced crystallization is not clear at present.

All of the pine species whose resin crystallized in the present tests belonged to the series Strobi of the pine subgenus Haploxylon. Of the non-reactive species in this series, *P. lambertiana* Dougl. (sugar pine) has not been successfully crossed with any other member in the series and does not grow well in the Northeast. *P. monticola* has been grown to a small extent in the Northeast and hybrids between this species and *P. strobus* have shown many desirable traits. However, the resistance of *P. monticola* to insect-induced resin crystallization was not exhibited in the control-pollinated hybrids that were tested. On the other hand, *P. peuce* appeared to be capable of transmitting its crystallization resistance to its interspecific progeny. Four of the five *P. peuce* X *strobus* hybrids tested were siblings from the same non-crystallizing female parent, and the resin from two of these did not crystallize. The same *P. peuce* female was also involved in the hybrid *P. peuce* X *griffithii,* the resin of which also resisted crystallization in the tests.

In the laboratory tests with live beetles on pine branches bearing 1-year-old conelets, several successful attacks occurred. Of four beetles placed on fresh branches of *P. strobus* on April 13, 1964, one beetle started to enter the base of a conelet almost immediately, and had completely disappeared in 3 hours. By the next morning, another beetle had entered the base of a peduncle. Successful attacks also were made on conelets of two *P. peuce* X *strobus* hybrids after the branches had been in the laboratory for 3 days. The resin of one of these hybrids crystallized readily in the standard test and also showed natural crystallization; the resin of the other hybrid did not crystallize under either circumstance. However, we cannot be sure that either the attacks or the behavior of the resins in these hybrids was strictly normal because of the possibility that the branches underwent physiological disturbances during the 3-day test period. Such disturbances, if they occurred, could have resulted in abnormal host-pest relationships. None of the attacking beetles emerged during the 2 weeks when the branches were kept under observation.

It is not known whether the insect-induced crystallization reaction of shoot resins indicates resistance to the white-pine cone beetle or not. However, the results suggest that at least two of the species (*P. monticola* and *P. peuce*), which should be used extensively in any breeding program for resistance to the white-pine weevil, may also contribute some degree of resistance to the white-pine cone beetle.

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